Approaches to Detecting Immunotoxic Effects of Environmental Contaminants in Humans

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Experimental animal studies indicate that environmental contaminants can have adverse effects on several organs and tissues of the immune system. Such effects are known to lead to increased host susceptibility to microbial infections and to compromised immunosurveillance mechanisms normally instrumental in the elimination of neoplastic cells and the prevention of autoimmune diseases. Evaluation of the potential risk environmental contaminants pose to the human immune system is currently accomplished via extrapolation of experimentally derived animal data to humans. Presently, this process requires that uncertainty factors such as interspecies differences and genetic variability be considered. Naturally, the process of risk assessment would be greatly facilitated if it were based on clinically relevant data derived from studying humans known to be exposed to environmental contaminants. However, the existing human data are scarce and often described as very limited in scope. To generate the much-needed human data we need to identify a set of clinically relevant immunologic end points that, when adequately standardized, can be incorporated easily into the design of prospective epidemiologic studies. *Key words*: environmental contaminants, immunotoxicity, testing approaches. — *Environ Health Perspect* 109(suppl 6):877–884 (2001).
http://ehpnet1.niehs.nih.gov/docs/2001/suppl-6/877-884tryphonas/abstract.html

Environmental contaminants including polychlorinated biphenyls (PCBs), dioxin [2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)], and polybrominated biphenyls; metals such as lead, mercury, and cadmium; and pesticides including hexachlorobenzene, mirex, dieldrin, and dichlorodiphenyltrichloroethane (DDT) are widely spread throughout the aquatic and the terrestrial ecosystems (*1,2*). The persistence of such chemicals in the environment, their bioaccumulation in living organisms, and their potential to induce adverse health effects, including effects on the immune system, cause concern to government regulators and to the public at large. These concerns are being addressed by several regulatory agencies at the national and international levels (*3–5*).

Chemical-induced adverse effects have been noted in a variety of life forms including marine mammals (*6*), birds (*7*), and in rodents and humans (*8*). Although a number of systems can be affected by environmental contaminants, experimental animal data indicate that the immune system is one of the most sensitive targets for chemical-induced toxicity, especially for the chlorinated compounds TCDD (*9,10*) and PCBs (*11–14*). Effects on the immune system include hematologic changes, a reduction in bone marrow cellularity, and thymic and splenic atrophy, which correlate with humoral and/or cell-mediated immunosuppression. Such effects may be manifested as reduced resistance to microbial infection (*15*), increased incidence of autoimmune disorders (*16*), and compromised immune surveillance mechanisms responsible for the clearance of neoplastic cells (*17*).

Chemical-induced immunotoxic effects are investigated within the discipline of immunotoxicology. This is presently accomplished with an array of validated immunologic tests in experimental animal models. Although immunotoxicology is a relatively young discipline, it has generated a large database in experimental animals (*18*). Data derived from several of these studies have been used in the assessment of potential risk levels for human exposures (*9,14*).

Risk assessment involves the process of extrapolating from experimental animal data to humans, and it considers several levels of uncertainty, which are factored in the final analysis (*19*). Ideally, the process of risk assessment would be biologically more meaningful if it were based on data derived from humans known to be exposed to environmental contaminants. Presently, such data are scarce and limited in scope, as only a few basic immunologic end points have been investigated in occupationally exposed workers or in cross-sectional studies of accidentally exposed cohorts. To improve the process of risk assessment, we need to enrich the human database. This entails identifying a number of clinically relevant immunologic end points that can be easily incorporated into well-designed epidemiologic studies. This article focuses on a review of current testing strategies and incorporates additional immunologic end points that may be useful in the investigation of potentially adverse chemical-induced immunomodulation in humans. Human data indicative of effects of environmental contaminants on the immune system are also discussed.

Immunologic Markers of Effect

The immune system is structurally and functionally complex (*20*). It consists of several tissues and organs strategically positioned throughout the body. Because of this complexity, multiple immunologic end points (markers of effect) must be examined before a comprehensive evaluation of the potential immunomodulatory effects of chemicals can be established.

Immunologic markers of effect include changes in several components of the immune system, such as shifts in the distribution of lymphocyte subpopulations and changes in other tissues caused by immune-mediated dysfunction, for example, signs of kidney failure caused by autoimmune kidney disease (*21*). Consequently, the use of markers with a high correlation to a particular immunotoxic end point is valuable in the identification of the presence of these health effects.

A number of testing schemes have been proposed for assessing humans exposed to immunotoxicants. These include *a*) a testing scheme proposed by the World Health Organization (WHO) for assessing immunotoxicity in all persons exposed to immunotoxicants (*18*), *b*) a screening panel of assays recommended by a working group organized by the U.S. Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry (*18*), *c*) a 2-tier approach proposed by a panel of scientists convened by the U.S. National Academy of Science (*22*), and *d*) a more recent 3-tier approach proposal for testing immune effects on humans (*23*). Although a number of similarities exist among the various proposals, basic differences exist among all the proposed schemes regarding the choice of immunologic end points. Common to all the proposed

This article is based on a presentation at the Workshop on Methodologies for Community Health Assessment in Areas of Concern held 4–5 October 2000 in Windsor, Ontario, Canada.

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Received 11 January 2001; accepted 11 May 2001.

schemes are tests for the routine examination of hematologic parameters, including blood counts and differential counts, phenotypic analysis of leukocytes and lymphocyte subsets by flow cytometry, quantification of total serum immunoglobulin (Ig) levels, and autoantibody titers (to rheumatoid factor, to the nucleic acid DNA, to mitochondria, etc.). In addition, proposals *a* and *b* include tests for serum clinical chemistry, and proposals *a* and *c* include tests for specific and nonspecific immunity as well as tests for cellular immunity. Last, proposals *c* and *d* include the quantification of levels of cytokines (basal and stimulated) in serum and *in vitro* activation systems. The scheme proposed by the U.S. National Academy of Science panel is by far the most complete scheme, as it encompasses all aspects of specific and nonspecific immunity and, to a very limited degree, autoimmunity and allergy. Unfortunately, none of the existing human studies on adverse immunologic effects of environmental contaminants has used any of the proposed schemes in its entirety. Consequently, the power of any single scheme to predict chemical-induced immunomodulation remains to be investigated. To further assist researchers in determining the type of immunologic end point(s) to be investigated in human epidemiologic studies, we summarize in the following sections relevant information regarding a number of key immunoassays. Where possible, we also present recent advances in this area. Lack of space prohibits a detailed discussion of immunologic methods. For such information the reader is referred to the several excellent publications on clinical immunology (*18,24*). In addition, chemical-induced hypersensitivity reactions and the development of autoimmunity in exposed individuals are two areas that are of increasing concern to human populations. However, both of these clinical entities have complex underlying mechanisms and require specialized methodologies for a proper diagnosis, both of which are extensively reviewed by Bigazzi (*16*) and will not be included in the present review.

General Parameters of the Immune System

Total white blood cell counts. Quantitative and morphologic investigations of total white blood cells (WBC) and differential counts of peripheral blood (PB) are basic investigations and have been included in most immunotoxicity studies. In experimental animal studies WBC counts correlate poorly with functional aspects of the immune system. Luster et al. (*25*) reported a concordance of only 43% between WBC counts and biologically relevant *in vivo* functional immune defects. However, in humans WBC counts have proven useful in signaling clinically relevant hematologic changes that may result in clinically identifiable autoimmune disorders of the blood cells, e.g., idiotypic thrombocytopenia, and various forms of leukemia (*24,26*). Both relative and absolute numbers of WBC are quantified. However, the consensus is that absolute numbers provide biologically more relevant information, as the use of percentages of cell types may mask some cytopenias or excessive numbers of a cell type, which would lead to falsely high/low numbers of a particular cell (*27*). The calculation of absolute counts takes into consideration the total WBC count, the percentage of total lymphocytes, and the percentages of each subset as follows:

$$\frac{\text{Absolute lymphocytes}}{\text{mm}^3} = \frac{\text{total WBC}}{\text{mm}^3} \times \frac{\left(\frac{\text{\% of total}}{\text{lymphocytes}}\right)}{100} \times \frac{\left(\frac{\text{\% of each}}{\text{lymphocyte subset}}\right)}{100}$$

In addition to the well-documented age-related fluctuations of WBC counts, several factors that influence the immune system may also cause fluctuations in the WBC counts: *a*) age, *b*) race, *c*) sex, *d*) pregnancy status, *e*) stress, *f*) coexistent disease or infection including HIV, *g*) nutritional status, *h*) lifestyle, *i*) tobacco smoking, *j*) certain medications, *k*) biologic response modifiers, and *l*) postoperative procedures It is, therefore, necessary that repeated counts of WBC be obtained over time. More important, these counts should be compared to normal age- and sex-matched control values obtained at the same time points as the experimental counts. Alternatively, the experimental values can be compared with existing historical control values (*24*).

Immunophenotyping of peripheral blood lymphocytes. Immunophenotyping of PB lymphocytes, using monoclonal antibodies directed to cell-surface markers and flow cytometric techniques, has become an important tool in the diagnosis of hematologic and immunologic disorders (*27*). In experimental animals enumeration of T-lymphocyte subpopulations was shown to be a sensitive end point, with a concordance of 83% between the number of T-cell subsets and biologically relevant *in vivo* effects (*25*). In humans the study of T-cell subsets has its clinical application in the characterization of T- or B-cell abnormalities, e.g., primary immune deficiency, and acquired T-lymphocyte subpopulation deficiencies, and in detecting atypical cell markers in peripheral blood lymphocytes, e.g., in many types of leukemia (*24,26*). As with the total WBC counts, determination of the various T-cell subsets and B cells should be expressed both as percent of lymphocytes and as absolute counts.

Immunophenotypic data in early childhood should be interpreted with caution, as during that period the immune system undergoes much expansion and maturation and is characterized by significant variations in both percent and absolute values of lymphocyte subpopulations (*28,29*). Thus, the observed immunologic immaturity of the young may be responsible for the reported increased susceptibility to infections during the first 5 years of life (*30*). Conversely, T-cell subsets in healthy adults whose immune systems have reached maturity are relatively stable (*31,32*). In adults the mean week-to-week variation in lymphocyte subpopulations measured over 13 weeks was less than 5% (*33*).

Because of the observed fluctuations in T-cell subsets in children, it is important that sequential analysis of experimental blood samples be performed and that results be compared with data derived from specific age- and sex-matched control groups or with valid historical control (normative) data. Normative data are available for human fetal and cord blood (*34,35*), for children (*28*), and for adults (*32*).

End Points for Humoral Immunity

Total serum immunoglobulin levels. Determination of total serum Ig levels (IgG, IgM, IgA, and IgE) in experimental animals has not proven useful, as significant effects on immune function are required before any effect on total serum Ig levels is observed. For example, no effects on serum Ig levels were observed in nonhuman primates exposed to low levels of PCBs, although significant effects were observed on the ability of the same animals to respond to a foreign antigen (*12*). Determination of Ig subclasses (IgA1 and 2 and IgG1–4) was a better predictor for immunotoxic effects of environmental contaminants (*36*). IgG subclass deficiencies have been associated with increased susceptibility to infections in the human upper respiratory tract, caused mainly by *Haemophilus influenzae* and *Streptococcus pneumoniae*, the development of allergy, asthma, and gastrointestinal disorders (*37,38*). In view of these findings, the WHO/International Union Of Immunological Societies (IUIS) working group has published a series of draft reports in which the determination of serum subclasses and the clinical interpretation of this end point have been discussed extensively (*39,40*). As with other parameters, Ig levels in exposed populations should be compared with levels in age- and sex-matched controls or to existing historical data (*41,42*).

Specific antibody levels to foreign antigens. The immune system is endowed with a large functional reserve capacity, and the changes in WBC numbers or shifts in lymphocyte subsets observed in many studies may not be accompanied by changes in immune function (*17*). It is important, therefore, that the functional capacity of the immune system be established. In experimental animals the immune functional capacity is evaluated using the various infectivity models that have been validated across several species (*6,43*). Results of such infectivity models correlate strongly with other immune function tests and are highly predictive of chemical-induced immunosuppression (*43*). However, for safety and ethical reasons, such an approach is not readily applicable to human studies. For these reasons human investigative studies are restricted to monitoring the incidence of infections in exposed and nonexposed human cohorts. Alternatively, the study subjects can be challenged with foreign antigens, followed by the determination of antigen-specific antibody levels in serum collected prior to antigenic challenge (baseline titers) and at weekly intervals postimmunization. In experimental animals this approach has been highly predictive of effects on humoral immunity (*12,25,43*).

The response to an antigenic challenge involves the sequential and tightly orchestrated interactions of functionally competent immune cells, including the macrophage/monocyte (antigen-processing and -presenting cells), and the activated T and B lymphocytes. Consequently, much information can be derived from challenging the host with foreign antigens. Clinically relevant information includes the ability of the host to respond to a foreign antigen (primary response) and to establish memory (secondary or anamnestic response). Establishing memory endows the host with the ability to respond to a second insult by the same antigen in a much shorter time relative to the primary response. Analysis of pre- and postimmunization levels of antigen-specific antibody levels in serum collected at weekly intervals can also be used to study the catabolic rate of the specific antibody and the ability of the immune system to switch from IgM to IgG isotypic class switching, both of which influence the level of detectable antibody to a given antigen (*44*).

The effects of environmental contaminants on the primary humoral immune response can be studied only when an antigen has met certain basic criteria. First, the antigen must be foreign to the host, i.e., no previous encounter. Second, it must be immunogenic. Finally, the levels of antigen used for eliciting an antigenic response should should not adversely affect the health of the subject.

An antigen that fulfills all the above criteria is bacteriophage phiX174. Injecting the subject once with phiX174 will elicit a primary response. A second injection 4 weeks later will produce a strong anamnestic response. This procedure allows for the determination of primary and anamnestic responses to the bacteriophage challenges and makes possible the concurrent measuring of the rate of clearance of the injected bacteriophage (*45*). Decreased bacteriophage clearance has been shown to be significantly delayed in immunodeficient patients (*45*). Immunization with bacteriophage has been conducted by several groups in different countries and has proven to be a harmless procedure (*44*).

Another potentially useful antigen is keyhole lympet hemocyanin (KLH). KLH is extracted from *Metathura crenulata* (keyhole limpets) collected from the sea in the wild (*46*). Both primary and anamnestic responses to this antigen can be monitored in humans (*47,48*). When this antigen is used to elicit a primary response, one should be aware of the possibility that humans may have been exposed to cross-reactive immunogens in the past, leading to detectable titers of natural antibodies. The levels and classes of natural antibodies can be measured prior to immunization and must be considered when results are interpreted. KLH, without the use of adjuvants, has been used extensively in experimental animal studies to test the effects of environmental chemicals on immune function (*49*). Clinically defined adverse effects due to KLH have not been reported in these studies.

In addition to the above-described antigens, the recombinant hepatitis B vaccine preparation that is used widely for protecting humans against hepatitis B infection may also be a potentially useful antigen (*50*). Use of such an antigen in immunotoxicity studies is limited because more than one injection of the antigen is required to obtain seroconversion, and the rate of response to this antigen diminishes with age (*51*). Furthermore, adverse effects including severe pancytopenia have been observed in humans vaccinated with this antigen (*52*). Currently, this vaccine is being applied to a study designed to evaluate the potential immunotoxic effects of ozone in a large number of adults (*53*). Such data will be helpful in ascertaining the usefulness of such a vaccine in determining chemical-induced immunosuppression.

The anamnestic immune response can be evaluated by determining serum antibody levels to antigens that humans are commonly vaccinated with, such as tetanus toxoid (TT) and diphtheria (*54*). The levels of protective antibody specific for these antigens in the population vary with age. According to the National Health and Nutrition Examination Survey conducted from 1988 to 1991, 69.7% of Americans ≥6 years of age had protective levels of tetanus antibodies (>0.15 IU/mL) (*55*). However, the rate decreased from 87.7% among those 6–11 years of age to 27.8% among those ≥70 years of age. Among children 6–16 years of age, 82.2% had protective levels of tetanus antibodies (*55*). Thus, it is important that baseline levels of antibodies to a specific antigen be measured prior to challenge regimes.

Both the primary and anamnestic immune responses are valuable for a comprehensive evaluation of the intrinsic naive and memory immune capacity of populations and should be investigated whenever possible. As is the case with other aspects of the immune system, the response to an antigen is genetically driven. This necessitates that a large number of exposed and nonexposed subjects be used.

End Points for Cellular Immunity

Lymphocyte transformation (tritiated thymidine incorporation). The ability of PB leukocytes to proliferate in response to several mitogens/antigens is tested using the lymphocyte transformation (^{3}H-thymidine incorporation) assay (LT) (*56*). The LT assay is considered an *in vitro* clinical correlate of delayed-type hypersensitivity to recall antigens. Several specific and nonspecific ligands can be used in this assay (Table 1). Plant mitogens such as phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) and bacterial products such as *Salmonella typhimurium* mitogen (STM) and *Staphylococcus aureus* Cowan 1 (SAC) have been used (*56*).

A useful extension of the LT assay is the quantitative analysis of Igs in the supernatants of cultured PB leukocytes with selected mitogens such as PWM, SAC, and STM or *S. paratyphi* B (SPB). By carefully selecting the set of mitogens, one can obtain valuable information regarding the type of cell affected by the chemical in question. Examples have been cited where a patient's PB leukocytes responded with IgM and IgA production to stimulation with PWM and *S. aureus* but failed to respond to SPB mitogen. The induction of Ig secretion *in vitro* by PWM and

Table 1. Substances used as stimulants in lymphocyte transformation (^{3}H-thymidine incorporation) assays.

Substance	Cell type activated
PHA	T lymphocytes
Con A	T lymphocytes
PWM	T-cell mitogen; B-cell mitogen through the release of soluble factors by T cells
S. aureus enterotoxin A	Potent T-cell mitogen
SAC	B cell
S. typhimurium	B cell
Killed SPB	B cell without T help

S. aureus is believed to be T-cell dependent, whereas SPB is believed to induce Ig synthesis without T-cell help. In the example above, two significant B-cell abnormalities are evident. First, it was not possible to stimulate B cells without T-cell help. Second, even when stimulated, the patient's B cells failed to produce IgG, pointing to a defect in the isotypic switch to IgG (*44*).

Delayed-type hypersensitivity. Determination of the delayed-type hypersensitivity response to recall antigens is a cost-effective method used to assess cell-mediated immune function in humans (*56*). The test involves the intradermal injection of antigen(s) and the measurement of erythema and induration at the injection site, peaking in intensity at 24–48 hr. The reaction is a lymphocyte- and macrophage-dependent delayed-type hypersensitivity response. A variety of antigens have been used, but the most practical for adult testing is a multitest kit (Multitest-CMI; Antigen Supply House, Northridge, California, USA) manufactured by The Institut Mérieux (Lyon, France). This kit contains *Candida albicans, Trichophyton mentagrophytes, Proteus mirabilis,* tuberculin purified protein derivative (PPD), streptococcus group C, diphtheria, TT, and a glycerin control. The multitest kit has been extensively standardized in adults, for whom hypoergic scores (2 standard deviations below the mean of healthy adults of the same sex and age) have been defined (*57*). Knicker et al. (*58*) tested 402 healthy adults 17–92 years of age and reported that only 0.5% were anergic to all antigens, with the remaining 95% having a reaction to one or more of the antigens tested. A review of all published data on delayed hypersensitivity response (DHR) in healthy adults was undertaken by Buckley (*59*). Results of such a review indicated that the proportion of humans responsive to various antigens was as follows: 53.3% to *C. albicans*; 75.5% to mumps, 43.5% to trichophyton, 37.6% to PPD, 38.3% to TT, and 20.4% to coccidioidin.

The multitest kit may not be suitable for infants or preschool children. Newborns under 6 weeks of age seldom have any DHR response, with infants 6–12 months of age having a high incidence of anergy (6.7%) (*59*). Normal, immunized 1-year-old children usually respond to at least one of the antigens candida, diphtheria, and tetanus, but do not respond to mumps, streptococcus, or trichophyton. Seventy-three percent of children 6 weeks to 12 years of age tested with candida and TT had at least one positive test (*60*). In addition, 6.8% of healthy preschool children are anergic, with a peak of 17% in the 3- to 4-year age group. The incidence of anergy is decreased in older children. Knicker et al. (*58*) found that none of 448 children 7–16 years of age were anergic. It should be noted here that mean values obtained for a specific population may vary from that of historical controls. Therefore, it is important that each study includes a nonexposed, age-matched control group.

Cytotoxic cells. A number of blood cells, including cytotoxic T lymphocytes, natural killer (NK) cells, and mononuclear phagocytic cells, are endowed with cytotoxic abilities and are thus very efficient in immunosurveillance mechanisms against neoplastic cells and viral infection. Attention has been focused on the NK cell because, like the macrophage/monocyte lineage of cells, its role as the first line of immune-mediated defense against viral and bacterial infections has been conclusively established. In immunocompromised hosts, there is a correlation between low NK cell activity and morbidity (*61*) or the incidence and severity of upper respiratory infections (*62*). Extreme susceptibility to herpes virus infection was reported in an individual without detectable NK cells (*63*).

NK cells are identified by the phenotype CD3-CD16$^+$ and/or CD56$^+$ (*64*). NK cell function is measured in a 4-hr ^{51}chromium (Cr)-release assay, whereby freshly isolated PB leukocytes (effector cells) and ^{51}Cr-labeled K562 cells (target cell) are co-cultured, and the release of label in culture supernatants is quantified. At least three concentrations of effector cells are added to a fixed number of ^{51}Cr-labeled K562 target cells. The amount of ^{51}Cr released is directly proportional to the level of NK cytotoxicity (*64*). Although the number of circulating NK cells is small (7–15% of circulating lymphocytes), they are functionally very efficient cells, as, unlike other cells whose function requires association with the major histocompatibility complex (MHC), NK cell action against certain malignant and virus-infected cells is MHC-unrestricted and nonspecific with respect to the type of cell targeted (*64*). In addition, NK cells produce numerous cytokines such as tumor necrosis factors α and β, interferons α and β, granulocyte-macrophage colony-stimulating factor, and interleukin-3 upon immune stimulation, all of which have a profound effect on immune reactivity (*64*).

Other Potentially Useful End Points

Thymic size. Chemical-induced toxicity of the thymus characterized by thymic atrophy has been observed with a number of environmental chemicals (*65*). TCDD, for example, targets the thymic reticular epithelium, resulting in lymphocyte depletion (*66*). A decrease in size or involution of the organ may thus be the first manifestation of environmental chemical-induced immunotoxicity and may be a useful indicator of immunotoxicity in the developing fetus following *in utero* exposure. The recently published series of reports by Hasselbalch and co-workers describes the application of sonography to the measurement of thymic size in preterm (born in weeks 24–32) infants and in term infants up to 24 months of age (*67–70*). The objective of this work was to correlate the size of the thymus in healthy infants with such clinical variables as breast-feeding status and illness. This technique when further validated may be useful in determining the effects of environmental chemicals on the level of immunologic maturity of the thymus in early childhood.

Quantification of cell-surface antigens and cytokines. Immune cells, normally in the resting phase, upon exposure to pathogens and chemicals become activated. The transition from the "resting" to the activated phase is accompanied by the expression of cell-surface antigens and the release of several cytokines (interleukins 1–12, interferon-γ, tumor necrosis factor, granulocyte–macrophage colony-stimulating factor, etc.) in the body fluids including serum (*71*). Agents such as viruses and chemicals, including environmental contaminants, alter the expression of cell-surface receptors and the release of cytokines, resulting in adverse effects on immune function. Therefore, quantification of these cytokines following activation of immune cells can generate useful information regarding the mechanism of action of environmental contaminants.

Factors That May Affect the Immune Response

The design of epidemiologic immunotoxicity studies is subject to the same rules that apply to any other epidemiologic study designs (*72*). However, unlike experimental animal studies in which interanimal variability can be minimized by using inbred animals that are uniform in age, studies involving human populations are subject to unavoidably large intersubject variability. This genetically controlled variability has obvious consequences for the capacity of the immune system to recognize foreign antigens and will ultimately influence the mechanism of chemical-induced immunotoxicity. In addition, a number of other factors may have an effect on the immune response, and these need to be taken into consideration in the design of studies involving humans (see "General Parameters of the Immune System").

For example, the inability of the developing fetus to recognize and react to a wide range of foreign substances and its increased susceptibility to long-term immunotoxic effects compared to that in adults is well documented (*30,73*). Changes observed in the thymus, the site of T-lymphocyte maturation, including a decline of serum thymic hormone activity and accelerated involution of the organ, are

age dependent (*74,75*). Increased frequencies of autoantibodies to nucleic acids, smooth muscle, mitochondria, lymphocytes, gastric parietal cells, Ig, and thyroglobulin have been well documented in older people (*73*). Pregnancy is characterized by profound changes in hormone levels that can have specific and nonspecific effects on the immune system. A decrease in peripheral blood lymphocytes in early pregnancy has been observed consistently (*76*).

Stress of various forms, including postoperative states, can affect the immune system. Because of the existing bidirectional pathways among the immune, endocrine, and neuronal systems, stress can have an effect on these systems. Changes in glucocorticoid levels are frequently cited as a result of this interaction (*77*). Fluctuations in WBC counts occur with glucocorticoid as a result of changes in hormone levels in the blood (*77*). Overall, stress has an immunosuppressive effect, causing changes in several functional aspects of the immune system.

Several other factors, including smoking and the use of nonprescription drugs, can also have profound effects on the immune system. Smoking, for example, causes a reduction in leukocyte counts (*78,79*), and nonprescription drugs such as aspirin can have a range of nonspecific immunologic effects, including inhibition of lymphocyte responses to mitogens and depression of neutrophil function (*80*). In view of the documented sensitivity of the immune system to a host of factors, basic considerations such as those listed below must be taken into account when designing human immunotoxicity studies:

- ensure that a set of selection procedures and adequate documentation of the study subjects are available
- ensure that the test and control populations are comparable in age, sex, socioeconomic factors, etc.
- ensure that the chemical(s) the study subjects are exposed to are adequately documented
- ensure that other factors, such as the presence of disease, especially HIV infection, or the use of medication that may influence the immune system, have been accounted for
- ensure that immunologic tests used to measure immune function are clinically relevant and validated
- ensure that levels (blood/fat) of the chemical(s) in question are available

Evidence for Chemical-Induced Immunomodulation in Human Studies

The limited data obtained from human epidemiologic studies suggest that the human immune system may be targeted by environmental contaminants (*17*). The available data are derived predominantly from monitoring humans exposed to a variety of chemicals in the workplace (occupational) or from cross-sectional studies in humans accidentally exposed to specific chemicals such as PCBs and dioxins. Furthermore, only a limited number of immunologic end points have been investigated in the majority of the documented studies. Given these limitations, available data regarding immune alterations associated with exposure to a variety of chemicals must be interpreted with caution.

Effects on the immune system have been reported in the Japanese (Yusho) and Taiwanese (Yu-Cheng) populations exposed accidentally to PCBs [Kanechlors (KC) 400 and 500], polychlorinated dibenzofurans, and quaterphenyls following the ingestion of contaminated rice oil (*81,82*). These findings indicated a compromised immune system in the exposed populations and were characterized by significant effects on both humoral and cellular aspects of immunity.

The Yusho patients exhibited a decrease in total serum IgA and IgM in the first year after the outbreak and a high frequency of respiratory infections (*83,84*). Similarly, the Yu-Cheng patients exhibited decreased total serum IgA and IgM, reduced T lymphocytes and T-helper/inducer cells, and reduced monocyte and polymorphonuclear leukocytes after 1 year of exposure (*85*). The incidence of positive skin reactivity to streptokinase/streptodornase (SK/SD) antigen mixture and to PPD antigens tested at 1 (SK/SD) and 4 years (PPD) after exposure was significantly lower in the Yu-Cheng patients than in controls (*86*). Of clinical significance is the observation that the percentage of patients showing a skin test response, as well as the size of the response, decreased with increased severity of the clinically observed PCB-induced dermal lesions and with increased PCB concentrations in whole blood (*86*). Yu-Cheng children had a higher frequency of bronchitis and influenza attacks at 6 months of age and a higher frequency of respiratory attacks and ear infections at 6 years of age, suggesting that their humoral immunity was compromised (*87*).

A higher incidence of colds and gastrointestinal (vomiting, abdominal pain) and dermatologic (eczema, itchy skin) manifestations were also observed in breast-fed infants born to women occupationally exposed to the KC-500 (chlorine content, 55%) and KC-300 (chlorine content, 43%) compared with infants born to nonexposed women. The incidence of these symptoms increased with increasing length of breast-feeding (*88*).

Epidemiologic studies of women who consumed contaminated fish from the Great Lakes indicated that the maternal serum PCB level during pregnancy was positively associated with the number and type of infectious illnesses suffered by the breast-fed infant, especially during the first 4 months of life during which maturation of the immune system is critical to the infant's health. The incidence of infections in the infant correlated strongly with the highest rate of maternal fish consumption and maternal blood PCB levels, suggesting that the observed changes to microbial resistance was induced by PCBs (*89,90*).

Shifts in T-lymphocyte subsets similar to those observed in experimental animals have also been noted in the infants of Inuit people in northern Quebec, Canada (*91*). A recent study by the same investigators reported that the incidence of otitis media was similar in both breast-fed and bottle-fed Inuit children but higher relative to that in other children residing in the southern Quebec area (*92*). Although no correlation was found between the incidence of otitis media in the Inuit children and maternal blood PCB concentrations, there was a significant correlation with levels of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and hexachlorbenzene (*92*). The incidence of otitis media was also reported to be higher in breast-fed than in bottle-fed infants in a recent study by Weisglas-Kuperus (*93*).

Weisglas-Kuperus et al. (*93–95*) investigated the effects of ambient levels of PCBs and dioxins on the immune system of breast-fed versus bottle-fed children in The Netherlands. A number of significant observations were made in the mother–infant pairs of breast-fed versus bottle-fed infants. These clearly indicated that humoral aspects of the infants' immune system were compromised. In addition, this study indicated that the observed effects might be due to exposure of the infants to PCBs and other contaminants, including TCDD, *in utero* or via breast milk. Similarly, children exposed to TCDD following an explosion at a herbicide factory in Seveso, Italy, were reported to have increased peripheral blood lymphoproliferative responses to mitogens 6 years after the explosion (*96*). A positive correlation between increased serum complement levels and the incidence of chloracne was noted in the same children (*97*). Similarly, positive correlations between increased levels of circulating T lymphocytes and adipose tissue TCDD levels were reported in 41 persons from Missouri, USA. TCDD levels in adipose tissue were the net result of occupational, recreational, or residential exposure. Serum IgA levels were increased in the exposed individuals compared to control (*98*).

Several organophosphate and organochlorine pesticides are known to affect the immune system (*99,100*). Altered levels of

serum Ig were observed in workers exposed to a combination of four organophosphate and organochlorine pesticides (malathion, parathion, DDT, and hexachlorocyclohexane) (*101*). Similarly, increases in serum IgG but decreases in serum IgM and complement C3 were reported in a study of 51 men exposed to chlorinated pesticides, compared to a control group (*102*). The clinical significance of the observed shifts in Ig and complement levels is not clear, as other relevant end points were not investigated. Impairment of neutrophil chemotaxis was reported for workers occupationally exposed to organophosphate pesticides compared to controls (*103*). The incidence of respiratory infections in the exposed workers was increased compared to the control.

A recent study of subjects living near the Aberdeen Pesticides Dumps Site in Aberdeen, North Carolina, reported an association with altered levels of certain immune markers, which were correlated with plasma DDE levels (*104*). Specifically, a greater number of Aberdeen residents had a low percentage of NK cells, increased IgM levels, and decreased lymphoproliferative responses to mitogens compared to residents of the comparison areas. Younger Aberdeen residents (18–40 years of age) and residents who lived in Aberdeen prior to 1985 when the pesticide plants were in operation had a 2- to 3-fold increased risk for developing herpes zoster (shingles) compared to residents of nearby communities (*104*).

Data derived from cohorts suggested that lead (Pb), mercury (Hg), and cadmium (Cd) may affect the immune system (*105*). The effects of Pb and Cd were predominantly on cellular aspects of the immune system (*106–109*), with humoral parameters remaining relatively insensitive (*110*). Conversely, exposure to Hg resulted in significant shifts in circulating total lymphocytes and their subsets (*111*) and Ig levels (*112,113*).

Finally, the possible association between immunotoxicity caused by environmental chemicals and the development of cancer is not well understood. Studies based on data derived from company and municipal death records suggest an association between occupational exposure to known immunotoxicants present in grain mills and higher incidence of neoplasms in the hemopoietic and lymphatic tissues (*99,100*). Similarly, an increase in the incidence of myeloid leukemias occurred among pesticide workers in Florida (*114*). A review of epidemiologic studies of humans exposed to pesticides performed from 1975 to 1991 revealed an increased risk of myeloproliferative disorders associated with exposure among manufacturers, applicators, and farmers (*115*). The recently published reports on areas of concern in the Great Lakes region (*1,2*) document the existence of a number of cancers of the lymphatic and hematopoietic system, such as non-Hodgkin lymphoma, Hodgkin lymphoma, and leukemia, for the Windsor area. The results summarized by Gilbertson (*116*) indicated that there was more than a 2-fold higher (226%) incidence rate for mortality from Hodgkin disease in females. The rates of morbidity from leukemia were significantly elevated in both males (33% higher) and females (44% higher) 45–74 years of age compared with the rates in the rest of the province. The incidence for acute respiratory infections, other diseases of the upper respiratory tract, pneumonia, influenza, and chronic obstructive pulmonary diseases including chronic bronchitis, emphysema, and asthma was significantly elevated in the Windsor area compared to the provincial rates and those of Hamilton, Ontario (*116*). These results indicate that immunosurveillance mechanisms responsible for the elimination of neoplastic cells may be compromised.

Concluding Remarks

A number of immunologic schemes have been proposed for application to the study of chemical-induced immunosuppression in human cohorts. The majority of the proposed end points, including determination of the total serum Ig classes and subclasses, quantification of peripheral blood leukocytes and T-lymphocyte subsets, the lymphoproliferative activity of peripheral blood leukocytes in response to mitogens, NK cell activity, and monocyte function can easily be investigated in *in vitro* systems using peripheral blood from humans known to be exposed to environmental contaminants, and are therefore noninvasive in nature. Others, such as the delayed-type hypersensitivity response to recall antigens, require intradermal injection of antigens and should be performed under medical supervision. The ultimate choice of end points to be investigated will depend largely on the age of the cohort. For example, in preschool-age children, data on the antibody response to antigens commonly used for vaccination and delayed-type hypersensitivity responses combined with data on the incidence of microbial infections, would be useful in determining whether the immune system is compromised relative to age- and sex-matched unexposed controls. In adults, determination of antibody production in response to specific antigens such as bacteriophage, KLH, TT, and hepatitis B vaccine would be desirable.

A number of the above-mentioned end points have been investigated in humans exposed to a number of environmental contaminants. The resulting data suggest that the human immune system is vulnerable to the immunotoxic effects of environmental contaminants and may have detrimental health effects. Therefore, investigators should be encouraged to incorporate a set of clinically valid end points into all future epidemiologic studies of cohorts known to be exposed to environmental contaminants. Guidelines already established for the design of epidemiologic studies in general (*105*) also apply to the design of immunotoxicity studies in human cohorts. In addition, several confounding factors known to affect the immune system must be considered in the statistical evaluation of all immunologic studies. For example, the presence of undiagnosed HIV infection, even in a small proportion of individuals included in a study population, may significantly affect the results and, consequently, the interpretation of data.

Finally, it should be emphasized that the normal immune system has a broad spectrum of reactivity and a great deal of reserve capacity. Consequently, the presence of a statistically valid correlation between the blood/adipose tissue levels of the chemicals investigated and the presence of clinically relevant outcomes should be investigated. Only then can one conclude with considerable certainty that the observed adverse immunologic effects occur as a result of exposure to the chemical(s) in question.

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